VIROLOGY 149, 247–250 (1986)

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Molecular Cloning and Characterization of Human Papillomavirus Type 7 DNA

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Received July 12, 1985; accepted November 25, 1985

Human papillomavirus type 7 (HPV-7) was first described in 1981 but so far could not be molecularly cloned. It has been found almost exclusively in hand warts of butchers. We have cloned the complete genome in pBR 322, established its physical map, demonstrated the colinear genome organization with HPV-18 and analyzed the degree of homology with other HPV types and bovine papillomavirus (BPV) types. In order to investigate whether HPV-7 might be a so far unidentified bovine virus, we screened 37 bovine tumor DNAs using Southern blot analysis for its presence, with exclusively negative results. From our data we conclude that the HPV-7 genome shows all characteristics of a papillomavirus genome and that its origin is most likely human. © 1986 Academic Press, Inc.

Over the last few years 40 distinct types of human papillomaviruses (HPV) have been identified. They have been found to be associated with a number of various tumors: benign proliferations like common warts and genital condylomas, as well as malignant ones like cancer of the uterine cervix and squamous cell carcinomas of the skin (reviewed in *1*). HPV-7 is one of the viruses found in common warts. It was first isolated in 1981 by Orth *et al.* (*2*) and independently by Ostrow *et al.* (*3*) from hand warts of butchers and slaughterhouse personnel. Since it had been noted earlier that butchers have a markedly increased incidence of hand warts, this professional group had been specifically investigated. HPV-7 was found in 25% of warts in such patients. This finding was consistent in different geographic regions as far apart as Eastern Europe and North America (*2, 3*). Unlike the other HPV types found in common warts (HPV 1–4), HPV-7 has so far been described exclusively in butchers with two exceptions (*13*). Therefore it was a reasonable hypothesis that HPV-7 is an animal virus transmitted to humans. However, no further evidence for this assumption could be established in the studies of Orth *et al.* (*2*) and Ostrow *et al.* (*3*). In contrast it was excluded that HPV-7 is identical with bovine papillomavirus types 1–4 (*2*). The aim of this study was to establish a cloned probe of HPV-7, which has not been reported before, to further characterize the genome and to gather more evidence regarding its natural host.

From a frozen sample of a hand wart of a butcher, we extracted total DNA according to standard procedures (*4*). The DNA was restricted with *Bam*HI and *Hind*III endonucleases. In an ethidium bromide stained agarose gel, distinct bands of viral DNA could be detected in the background of cellular DNA (not shown). Their molecular weights corresponded to the published data of HPV-7: 7.8 kbp for *Bam*HI digest, 4.0 and 3.8 kbp for *Hind*III digest (*2*). In Southern blot hybridization under conditions of low stringency (T_m-30°) (*5*) these bands were recognized by a ^{32}P-labeled HPV-6b probe. The two *Hind*III restriction fragments of HPV-7 DNA were isolated from an agarose gel, ligated to pBR 322, and transfected into *Escherichia coli*. HPV-7 DNA bearing recombinants were identified by colony hybridization (*6*) with labeled HPV 6b. Two clones were identified each carrying one of the two *Hind*III frag-

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0042-6822/86 $3.00

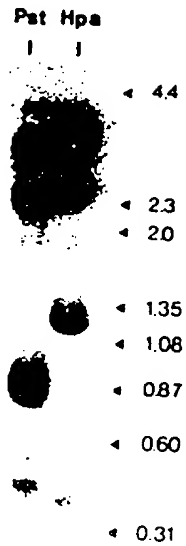

FIG. 1. *PstI* and *HpaI* restricted HPV-7 DNA isolated from virus particles (*2*) hybridized with ^{32}P-labeled cloned HPV-7 DNA. The molecular weights of the restriction fragments of the two isolates are identical and the cloned probe recognizes all fragments.

ments. These isolates were nick-translated with ^{32}P and hybridized under conditions of high stringency (T_m-20°) (Fig. 1) to the DNA of an earlier isolate from purified virus particles of HPV-7 described by Orth *et al.* (*2*). The cloned HPV-7 DNA is identical in the restriction endonuclease cleavage pattern with the DNA isolated from virus particles (*2*) suggesting that the complete genome is represented by the two *Hin*dIII clones. We established a physical map of the clones and analyzed their orientation by comparison of their endonuclease cleavage patterns with the pattern of DNA isolated from virus particles, which is not interrupted at the *Hin*dIII sites (Fig. 2). By hybridization of cleaved HPV-7 DNA with five labeled subgenomic fragments of HPV-18 (*7*) under conditions of low stringency (T_m-35°), the colinear genome organization of the two viruses was demonstrated (Fig. 3). From these hybridization data we concluded that the position 1 of the HPV-7 genome as first defined in BPV-1 by the single *Hpa*I restriction site (*8*) was situated next to the *Ava*II restriction site separating fragment B and D (Fig. 2). In order to define position 1 in HPV-7 105 bp of the nucleotide sequence around this *Ava*II restriction site were determined by the Sanger dideoxynucleotide technique (*9*) and aligned with the sequences of HPV-16 (*10*) and HPV-11 (*11*) (Fig. 4). The sequenced fragment represents part of the noncoding region of the HPV-7 genome upstream of the E6 open reading frame which defines the beginning of the early region of papillomaviruses. The mentioned *Ava*II restriction site is therefore situated at position 44 (Fig. 4).

To determine the degree of homology with other papillomaviruses we hybridized labeled HPV-7 DNA to equal amounts of cloned genomes of all papillomaviruses available to us using different conditions of stringency (42° in 0.82 *M* Na$^+$ and 20, 35,

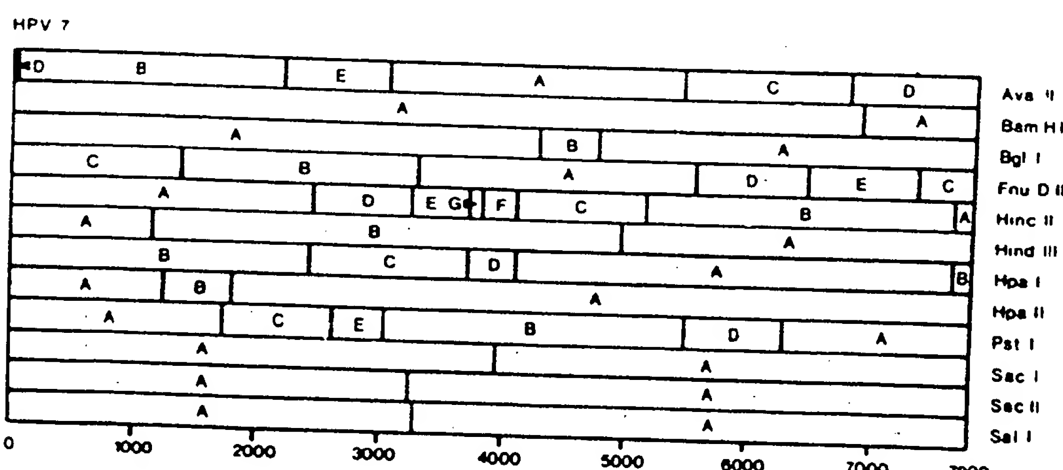

FIG. 2. Restriction endonuclease cleavage sites in HPV-7 DNA. The circular genome has been opened at position 1 (Fig. 4).

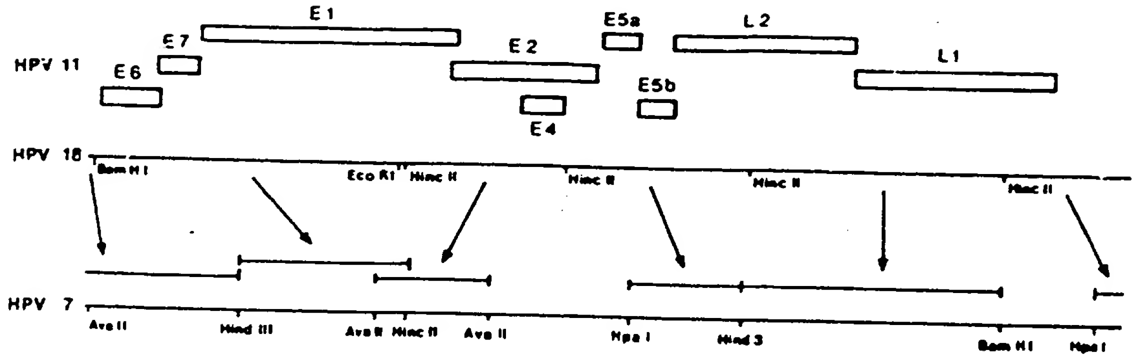

FIG. 3. Colinear genome organization of HPV-7 and HPV-18. Five HPV-18 subgenomic fragments were labeled separately. In the DNA-DNA hybridization under conditions of low stringency (Tm-35°), they recognize the HPV-7 fragments indicated by arrows. The arrangement of open reading frames is shown in HPV-11 aligned to HPV-18.

or 50% formamide giving T_m-40°, -30°, or -20°, respectively) (12). All papillomaviruses tested (HPVs 1-18, 21-25, 31; BPVs 1 and 3-6) hybridized with the HPV-7 probe at T_m-40°. At T_m-30° and T_m-20° none of the BPV types and only 9 of 24 HPV genomes tested were recognized (Fig. 5). It is remarkable that the best hybridization was obtained with HPV types 6, 11, 16, and 31, which are present in mucosa. HPV-8 which was isolated from a skin tumor is an exception to this observation. Other HPV types occurring in epidermal cells reacted only weakly. Preliminary sequence data encompassing the complete noncoding region, the E6, E7 and part of the E1 open reading frames support the hybridization data by showing a close relationship of HPV-7 to other sequenced human papillomaviruses. To analyze further whether HPV-7 may represent an unidentified BPV (bovine papillomavirus), a series of 37 DNA samples from bladder and intestinal papillomas and carcinomas of cattle were screened for HPV 7 DNA. In Southern blots under conditions of high stringency (T_m-20°), however, no HPV-7 specific signals could be detected.

HPV-7 meets the criteria for classification as a papillomavirus: colinear genome organization and sequence homology with other papillomavirus types, as well as detection of its DNA in virus particles. The question concerning the natural reservoir of the virus is so far unresolved. In an attempt to clarify what factors are involved in the unique host restriction to butchers, it was shown that HPV-7 is not identical with any of the known BPVs and that it does not occur in common papillomas and cancers of cattle. Therefore it is most probably not a bovine virus. We cannot ex-

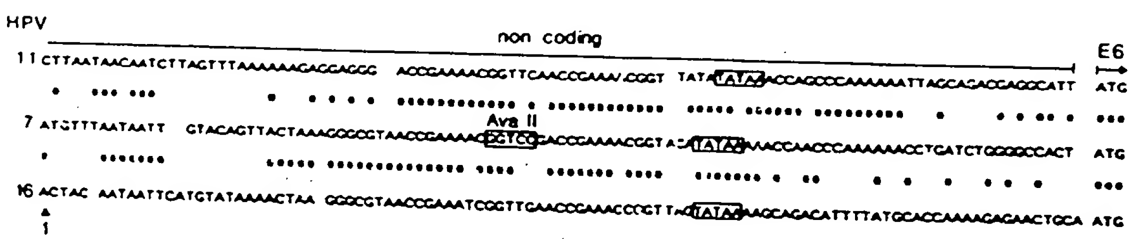

FIG. 4. DNA sequence of a 105-bp fragment of the HPV-7 genome aligned to the sequences of HPV-16 and HPV-11. Identical bases are marked by asterisks, the TATAA-sequences as well as the *AvaII* restriction site separating fragment B and D (Fig. 2) in HPV-7 are boxed. For sequencing the subgenomic fragment between this *AvaII* restriction site and the *HindIII* restriction site around position 1150 (Fig. 2) was cloned into phage M13 mp 18. The nucleotide sequence of the opposite strand including the *AvaII* restriction site was determined using a synthetic primer and a M13 mp 19 clone carrying the *HindIII* A restriction fragment.

HPV	3	5	6	8	11	13	16	18	31
Tm-30°	+	+	++	++	++	+	++	+	++
Tm-20°			+	++	+		++		+

FIG. 5. ^{32}P-labeled HPV-7 DNA was hybridized at Tm-20° and Tm-30° to the cloned papillomavirus genomes indicated. The intensity of the hybridization signals is given with + or ++. The intensity indicated by ++ is at least 50 times weaker, the intensity indicated by + at least 250 times weaker than with the homologous HPV-7 probe as estimated by different exposure times of the autoradiogram.

clude that HPV-7 is an animal virus, particularly as no porcine papillomaviruses have been identified so far, pork being the type of meat handled second most commonly by butchers. A study of HPV-7 infection in Jewish or Muslim butchers who never handle pork would help to elucidate this question. However, the fact that HPV-7 has so far only been found in humans, recently also in individuals without known animal contact (*13*), together with the pronounced cross-hybridization with some human papillomavirus types strongly suggests that HPV-7 is indeed a human virus. A series of 654 human tumor biopsies has recently been analyzed with the cloned HPV-7 probes by Southern blot analysis (*13*). Except for the mentioned two cases of extensive verrucosis no HPV-7 infections were demonstrated in persons without known animal contact. A similar problem of an unknown virus reservoir is encountered in epidermodysplasia verruciformis, a rare genetic disorder characterized by multiple warts and malignant skin tumors caused by HPV types that have never been detected in healthy persons (*13*). One could speculate that these HPV types lead to subclinical infections in healthy individuals, or are present in a latent state. Only specific host factors, like immunosuppression, might give rise to clinically apparent infections. In the case of HPV-7, the handling of slaughtered animals may be the factor leading to the activation of latent infection by the sustained contact with animal fluids which may contain immunosuppressive factors or substances causing skin maceration thus providing a milieu favoring locally virus replication resulting in an increased incidence of hand warts. This, however, does not seem to lead to general immunosuppression since butchers are not particularly susceptible to other viral infections. In addition no cases of HPV-7 infection in immunosuppressed patients, relatives of butchers or meathandlers or other persons with frequent animal contacts, like farmers or fishermen, have been identified although numerous materials also from cancer patients undergoing chemotherapy, renal transplant recipients, as well as from AIDS patients are routinely examined in our laboratory.

ACKNOWLEDGMENTS

We are grateful to H. zur Hausen for helpful suggestions. The work was supported by the Deutsche Forschungsgemeinschaft (SFB 31: Tumorentstehung und -entwicklung).

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